Claims

1. A method for the detection of relative positions in space of centers (x,y,z) of immobilized particles of a spatially encoded beaded or granulated matrix comprising said immobilised particles, wherein said particles comprise an optically detectable label,

said method comprising the step of recording of at least two 2D-projections of the particles,

said method optionally comprising the further step of determining, on the basis of the relative positions in space of centers (x,y,z) of immobilized particles,

a) the distance matrix for individual beads, or

b) a set of geometrical figures, such as triangles, defined by the relative positions in space of centers (x,y,z) of the immobilized particles embedded in said beaded or granumated matrix.

2. The method according to claim 1 wherein 3 2D-projections are recorded along 3 orthogonal axis x, y and z to generate 3 sets of 2D-coordinates (y,z), (x,z) and (x,y), respectively, from which the 3D-coordinates (x,y,z) of particle centers can be derived.

3. The method according to claim 1, wherein a plurality or stack of 2D projections are generated by confocal or focal microscopy to recreate the 3D image matrix of the bead from which the relative particle position (x,y,z) in space can be determined.

4. The method of any of claims 1 to 3 employing at least one focussed scanning laser for detection of relative positions in space of centers (x,y,z) of immobilized particles and laminar fluidics for bead manipulation.

5. The method of claim 4 in which the coordinates x and y of a particle position are determined by fast scanning two orthogonally aligned lasers over two cross sections

of the moving bead while the z coordinate is determined by the time of flight of the bead at known flow rates.

6. The method of any of claims 1 and 2 in which the coordinates x and y of a particle position are determined by using a single laser and a rotating mirror that via 2 or 3 geometrically arranged static mirrors reflects the laser beam along 2 or 3 orthogonal axes.

7. The method of any of claims 1 to 6, wherein said method comprises the steps of identifying an individual bead, b_q, of a plurality of beads, $B = (b_1, b_2, ..., b_H)$, where $1 \leq q \leq H$, and H being the number of beads, wherein H is preferably in the range of from 10^3 to 10^7, by a method for comparing a) a set of geometrical figures, such as triangles, for a bead to be identified, with b) the set of geometrical figures, such as triangles, for all beads of a population of beads comprising the bead to be identified, wherein said method comprises the steps of

1. providing a plurality of spatially encoded beads, B,

2. obtaining at least one orthogonal pair of images, $(I_{h,x,z}, I_{h,y,z})$, of each bead, b_h, where h = 1, 2, ..., H, of said plurality of distance encoded beads, B,

3. deriving from each of said at least one orthogonal pair of images, $(I_{h,x,z}, I_{h,y,z})$, the set, C_h, of possible sets of three-dimensional particle positions represented by x, y, and z image pixel values for each bead, b_h,

   $C_h = (c_{h,1}, c_{h,2}, ..., c_{h,Eh})$, where $c_{h,e} = (x_{h,f,e}, y_{h,f,e}, z_{h,f,e})$,

   where f = 1, 2, ..., F_h, and F_h being the number of particles of bead b_h, and e = 1, 2, ..., E_h, and E_h being the number of possible sets of three-dimensional particle positions for bead b_h,

4. deriving for each set of possible sets of three-dimensional particle positions one distance matrix

$$D_{h,e} = \begin{vmatrix} 0 & d_{h,e,1,2} & d_{h,e,1,3} & \cdots & d_{h,e,1,Fh} \\ d_{h,e,2,1} & 0 & d_{h,e,2,3} & \cdots & d_{h,e,2,Fh} \\ d_{h,e,3,1} & d_{h,e,3,2} & 0 & & \cdots \\ \cdots & \cdots & & \cdots & \\ d_{h,e,Fh,1} & d_{h,e,Fh,2} & \cdots & & 0 \end{vmatrix}$$

where $d_{h,e,i,j}$ = integer($[(x_{h,e,i}-x_{h,e,j})^2+(y_{h,e,i}-y_{h,e,j})^2+(z_{h,e,i}-z_{h,e,j})2]^{½}$), where i = 1,2,...$F_h$, and j = 1,2,...,F_h ,

5. deriving for each distance matrix, $D_{h,e}$, the full set of derivable triangles, $T_{h,e}$ = ($t_{h,e,1}$, $t_{h,e,2}$, ..., $t_{h,e,Ghe}$), each triangle being represented by its three side length,

$T_{h,e}$ = [$t_{h,e,1}$, $t_{h,e,2}$,..., $t_{h,e,Ghe}$] = [($d_{h,1,2}$, $d_{h,1,3}$, $d_{h,2,3}$), ($d_{h,1,2}$, $d_{h,1,4}$, $d_{h,2,4}$),...,($d_{h,(Fh-2),(Fh-1)}$, $d_{h,(Fh-2),Fh}$, $d_{h,(Fh-1),Fh}$)],

$G_{h,e}$ being the total number of derivable triangles from distance matrix, $D_{h,e}$,

6. generating a subset, U, of all triangles, T, derived for the full set of beads, B, said subset of triangles comprising all different triangles derived for the full set of beads,

U = (u_1, u_2, ..., u_W),

where $u_i \neq u_j$, for $i \neq j$, and i = 1, 2, ..., W, and j = 1, 2, ..., W, and W being the total number of different triangles derived for the full set of beads, B,

7. generating a look-up table, L, that for every triangle, u_r , where r = 1, 2, ..., W, gives the subset, A_r , of the full set of beads, B, for which subset of the full set of beads at least one of its derived sets of triangles comprises u_r ,

L = [(u_1, A_1), (u_2, A_2), ..., (u_W, A_W)],

8. obtaining at least one orthogonal pair of images ($I_{q,x,z}$, $I_{q,y,z}$) of the bead, b_q, to be identified,

9. deriving from said at least one orthogonal pair of images ($I_{q,x,z}$, $I_{q,y,z}$) the full set of possible sets of three-dimensional particle positions,

$$C_q = (c_{q,1}, c_{q,2}, \dots c_{q,Eq}),$$

10. deriving for each of said sets of possible sets of three-dimensional particle positions one distance matrix

$$D_{q,e} = \begin{vmatrix} 0 & d_{q,e,1,2} & d_{q,e,1,3} & \cdots & d_{q,e,1,Fq} \\ d_{q,e,2,1} & 0 & d_{q,e,2,3} & \cdots & d_{q,e,2,Fq} \\ d_{q,e,3,1} & d_{q,e,3,2} & 0 & & \cdots \\ \cdots & \cdots & & \cdots & \\ d_{q,e,Fq,1} & d_{q,e,Fq,2} & \cdots & & 0 \end{vmatrix}$$

11. deriving for each distance matrix, $D_{q,e}$, the full set of derivable triangles, $T_q = (t_{q,e,1}, t_{q,e,2}, \dots, t_{q,e,Gqe})$, each triangle being represented by its three side length,

$$T_q = [t_{q,e,1}, t_{q,e,2}, \dots, t_{q,e,Gqe}] = [(d_{q,1,2}, d_{q,1,3}, d_{q,2,3}), (d_{q,1,2}, d_{q,1,4}, d_{q,2,4}), \dots, (d_{q,(F-2),(F-1)}, d_{q,(F-2),F}, d_{q,(F-1),F})],$$

12. finding for each of said triangles of said set of triangles, T_q, derivable from bead b_q the corresponding set, B_q, of subsets of beads according to said look-up table, L, for which at least one of its derived sets of triangles comprises each of said triangles of said set of triangles, T_q, derivable from bead b_q,

13. registering for each of the beads of said subset of beads, B_q, the number of triangles contained in T_q, and thereby

14. identifying bead b_q as the bead of said subset of beads, B_q, that has the highest number of triangles contained in T_q.

8. The method or polymer matrix according to any of the preceding claims, wherein the optically detectable particles comprise fluorescence labelled polyethylene-grafted polystyrene microspheres.

9. The method of claim 7, wherein the diameter of the microspheres are from 10 to 30 micrometers, such as e.g. 10 micrometers, 20 micrometers, or 30 micrometers.

10. A method for distance matrix determination of at least one spatially encoded beaded or granulated matrix comprising a plurality of spatially immobilised particles comprising an optically detectable label, said method comprising the steps of

providing at least one beaded or granulated polymer matrix,

providing at least one device for recording and storing at least one image of the at least one bead, said device comprising

at least one source of illumination,
at least one flow system comprising a flow cell comprising an imaging section
at least one pulse generator,
at least one image intensifier,
at least one CCD camera,

activating at least one source of illumination,

introducing the at least one encoded bead comprising a plurality of particles into the flow cell comprising an imaging section,

recording at least one image of the at least one bead by sending substantially simultaneously a pulse generated by a pulse generator to both a) the at least one image intensifier, and b) the at least one CCD camera capable of recording said at least one image, and determining for individual beads a distance matrix based on said at least one image obtained for each bead.

11. The method of claim 10, wherein individual beads are determined according to the method of claim 7.

12. The method of claim 10, wherein the method steps are repeated for each individual bead entering the flow cell.

13. The method of any of claims 10 and 11 comprising the further step of detecting a bead entering the flow cell by using a photo-sensor.

14. The method of any of claims 10 to 12, wherein the pulse generator is activated by the activation of the photo-sensor, and wherein the photo-sensor is activated by an encoded bead entering the flow cell.

15. The method of any of claims 10 to 14 comprising the further step of storing the at least one image on a data storage medium.

16. The method of any of claims 10 to 15, wherein two CCD cameras and two image intensifiers are employed for recording the at least one image of the at least one bead.

17. The method of any of claims 10 to 16, wherein the source of illumination comprises a continuous wave laser capable of illuminating the imaging section of the flow cell.

18. The method of any of claims 10 to 17, wherein the photo-sensor for detecting the entry of an encoded bead into the imaging section of the flow cell comprises an optical objective for focussing said imaging section of said flow cell onto the photo-sensitive area of said photo-sensor, wherein said optical objective of said photo-sensor comprises a fluorescence filter capable of blocking the light of said laser, and wherein said fluorescence filter transmits the fluorescence emission from the particles.

19. The method of any of claims 10 to 18, wherein the at least one CCD camera for recording the at least one image of the at least one encoded bead comprises at least one gated image intensifier for amplifying the fluorescence emission from the encoded bead, and wherein each of said image intensifiers comprises at least one optical objective for focussing said imaging section of said flow cell onto the photo-sensitive area of each image intensifier, and wherein each optical objective comprises a fluorescence filter for blocking the light of said laser, and wherein the fluorescence filter transmits the fluorescence emission from the particles.

20. The method of any of claims 10 to 19, wherein a plurality of encoded beads are provided and wherein multiple distance matrices are determined based on individual distances recorded for individual beads.

21 The method of claim 20, wherein a set of multiple distance matrices is determined for a subpopulation of the beads based on more than one set of individual distances recorded for the subpopulation of beads.

22. The method of any of claims 20 and 21, wherein each distance matrix is recorded individually.

23. The method of any of claims 10 to 22, wherein at least one image of each bead is recorded per second.

24. The method of any of claims 10 to 23, wherein a total of more than 4000 beads are recorded per hour, such as more than 5000 beads per hour, for example more than 10000 beads per hour, such as more than 15000 beads per hour, for example more than 20000 beads per hour such as more than 25000 beads per hour, for example more than 30000 beads per hour such as more than 40000 beads per hour, for example more than 50000 beads per hour such as more than 60000 beads per hour, for example more than 70000 beads per hour, such as more than 80000 beads per hour, for example more than 90000 beads per hour such as more than 100000 beads are recorded per hour.

25. The method of any of claims 10 to 24, wherein the optical power of the laser is in the range of from 1 mWatt to preferably less than about 200 mWatt, such as about 10 mWatt, for example about 50 mWatt, such as about 100 mWatt, for example about 150 mWatt.

26. The method of any of claims 10 to 25, wherein the wave length of the laser is in the range of from about 450 nm to preferably less than 700 nm, such as about 500 nm, for example about 600 nm.

27. The method of any of claims 10 to 26, wherein the exposure time of the image intensifiers is preferably less than about 1 millisecond, such as less than 0.5 millisecond, for example less than 0.1 millisecond.

28. The method of any of claims 10 to 27, wherein the exposure time of the CCD cameras is preferably less than about 1 millisecond, such as less than 0.5 millisecond, for example less than 0.1 millisecond.

29. The method of any of claims 10 to 28, wherein the response time of the photosensor is preferably less than about 1 millisecond, such as less than 0.5 millisecond, for example less than 0.1 millisecond.

30. The method of any of claims 10 to 29, wherein the flow rate of the beads through the flow cell of the flow system is preferably more than 0.01 meter per second, for example more than 0.1 meter per second, such as more than 1 meter per second.

31. The method of any of claims 10 to 30, wherein the dimensions of the imaging section is preferably less than 1 milliliter, such as less than 0.5 milliliter, for example less than 0.1 milliliter, such as less than 0.05 milliliter, for example less than 0.01 milliliter, such as less than 0.005 milliliter, for example about or less than 0.001 milliliter.

32. The method of any of claims 10 to 31, wherein the flow cell is preferably made from a material which does not absorb the illumination light from the source of illumination and/or the emission light emitted from the particles, preferably quartz or a suitable transparent polymer.

33. The method of any of claims 10 to 32, wherein the size distribution of the beads is in the range of from 0.1 millimeter to preferably less than 2 millimeter, such as about 0.5 millimeter, for example about 1 millimeter, such as about 1.5 millimeter, and independently thereof, wherein the diameter of the paraticles is preferably less than 30 micrometer, for example less than 20 micrometer, such as less than 15 micrometer, for example in the range of from 5 to 15 micrometer.

34. The method of claim 33, wherein the majority of the beads, such as more than 75% of the beads, for example more than 90% of the beads, are in the range of from 0.5 millimeter to 1 millimeter, and independently thereof, wherein the diameter of the particles is in the range of from 5 to 15 micrometer.

35. The method of any of claims 10 to 34, wherein the optically detectable label is selected from the group consisting of light reflecting particles, light absorbing particles, dyes, fluorescent particles, and autofluorescent particles.

36. The method of any of claims 10 and 11, wherein the distance matrix for an individual bead is initially determined by a method comprising the steps of

determining for each particle of the encoded bead the 2D coordinates in the XZ-plane and in the YZ-plane, thereby generating a first set of data and a second set of data,

combining the first set of data and the second set of data and thereby obtaining 3D coordinates for each particle,

calculating the distance matrix as the full set of distances between particles for which preferably only one set of 3D coordinates is obtained.

37. The method of claim 36 comprising the further steps of

comparing the Z-coordinates of different particles within each bead, and

selecting particles wherein the difference between Z-coordinates is less than a predetermined threshold value, delta-Z,

pairwise grouping the selected particles according to delta-Z values,

maintaining the X-coordinate and the Z-coordinate for each of the pairwise grouped particles, and

switching the Y-coordinate between pairwise grouped particles, thereby obtaining an alternative set of 3D coordinates from which an alternative distance matrix can be calculated.

38. A method for identifying individual beaded polymer matrices in a composition comprising a plurality of such beaded polymer matrices, said method comprising the steps of

i) determining a distance matrix for individual beads,
ii) using the method of claim 7 for deriving from each of the distance matrices generated in step i) all of the possible geometrical figures, such as triangles, which can be generated by connecting particle coordinates with straight lines, and
iii) recording and storing the set of geometrical figures for each bead of the composition to be identified,
iv) selecting a subset of beads,
v) identifying one or more of the selected beads on the basis of a comparison of the set of possible geometrical figures of said bead(s) with all sets of possible geometrical figures recorded for the composition recorded in step iii).

39. The method of claim 38, wherein the geometrical figures are triangles.

40. The method of any of claims 38 and 39, wherein each bead comprises 3 or 4 spatially immobilised particles.

41. A method for identifying at least one individually identifiable, spatially encoded, bead in a composition comprising such beads, said method comprising the steps of

i) determining the unique, spatial position of three or more particles in the at least one bead to be identified,

ii) deriving from the positions, a matrix of the distances between the three or more particles,

iii) deriving from the matrix, a set of all possible triangles defined by the three or more particles, optionally by using the method of claim 7,

iv) identifying said at least one individually identifiable, spatially encoded bead based on a comparison of the set of possible triangles with all sets of possible triangles capable of being stored.

42. A method for recording individual reaction steps involved in the step-wise synthesis of a chemical compound on a beaded polymer matrix, said method comprising the steps of

a) spatially immobilizing a plurality of particles in polymer beads or granulates,
b) isolating, preferably by automated selection, at least a subset of the spatially encoded beads or granulates provided in step a), and
c) recording and storing a distance matrix or a geometrical figure derivable from the distance matrix for each bead or granule, said distance matrix or geometrical figurde being preferably generated by the method of any of claims 1 to 7,
d) stepwise synthesising chemical compounds on functional groups of the encoded beads or granules, wherein the identy of each bead or granule is recorded and stored for each reaction step, and
e) obtaining for each bead a record of individual reaction steps.

43. A method for identifying a chemical compound being synthesised on a beaded polymer matrix, said method comprising the steps of

a) performing the recording method of claim 42,
b) selecting beaded polymer matrices or granules of interest by using an assay or a diagnostic screen selective for the chemical compound having been synthesised on the beaded polymer matrix,
c) recording the distance matrix for each of the beaded polymer matrices selected in step b),
d) comparing the distance matrix recorded in step c) with all of the distance matrices recorded and stored in step c) of claim 100,

thereby obtaining information about the identity of the selected bead,

e) identifying for each selected bead the sequence of individual steps having lead to the synthesis of the chemical compound, and

f) identifying, based the sequence of individual steps the chemical structure of the compound.

44. The method of any of claims 41 to 43, wherein said plurality of particles comprise a fluorescently detectable marker.

45. The method of claim 44, wherein said fluorescently detectable marker is detectable by two photon fluorescence microscopy.

46. The method of claim 44, wherein said fluorescently detectable marker is detectable by one photon fluorescence microscopy.

47. The method of claim 44, wherein said fluorescently detectable marker is a UV or visible light-excitable microspheres.